REDUCTION OF ANDROSTENONE PERCEPTION IN PAN-FRIED BOAR MEAT BY DIFFERENT MASKING STRATEGIES

Running title: Reduction of androstenone perception in meat from entire pig

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Abstract

BACKGROUND

Consumers highly sensitive to androstenone will probably reject meat from entire male pigs, which tends to have high levels of this hormone. To avoid this, the effect of different masking strategies (sprinkling with mixed spices or fennel, marinating and breading with garlic-parsley or curry) on the sensory parameters of pork loin chops obtained from entire animals with high levels of androstenone (AND) (1.0-2.9 mg kg $^{-1}$ AND in fat) and castrated animals (< 0.4 mg kg $^{-1}$ AND in fat), both with low levels of skatole (SKA) (<0.1 mg kg $^{-1}$ SKA in fat) was investigated.

RESULTS

The garlic-parsley breadcrumbs led to the highest reduction in the perception of AND compared the other masking strategies used, and preserved the juiciness of the product. There was a negative correlation between AND and fat content.

CONCLUSION

AND odor and flavor can be reduced in meat from entire male pigs by using suitable strategies, the best strategy being the garlic-parsley breadcrumbs.

Keywords: strategies, masking, boar taint, androstenone, entire male pigs.

INTRODUCTION

Boar taint is an unpleasant odor and flavor of the meat from some entire male pigs, which is mainly associated with the presence of two compounds: androstenone (AND) and skatole (SKA). AND (5α-androst-16-ene-3one), a pheromone produced in the testes, has a urine-like odor, while SKA (3-methylindole), which is a breakdown product of the amino acid tryptophan in the large intestine, has a fecal-like, naphthalene odor. [1] SKA is perceived by a majority of consumers, while the ability to perceive AND varies among subjects. [2] In most EU countries, entire male pigs are castrated to avoid boar taint in pork.[1] However, there is increasing pressure to seek more humane alternatives to surgical castration, and do more meat from entire animals is now available in the marketplace.[3]

Consumers that are highly sensitive to AND will probably reject meat from entire male pigs with excess levels of AND and SKA.[4] However the correlation between liking or disliking AND and SKA depends not only on consumers' olfactory acuity, but also on others factors such as the type of product, cooking conditions and serving temperature.[5]

Several studies have focused on alternative outlets for boar tainted meat, e.g. bacon,[6] cooked ham,[7] dry cured ham,[8] and dry cured sausages.[2] The results of AND perception vary and depend on the temperature of consumption, AND and SKA levels and the strategy used to mask them. Some masking methods, such as using spices may be considered of interest since, historically, they were used because they extended the shelf life of food by inhibiting spoilage-causing microorganisms and masking off-flavor

compounds.[9] Some studies have shown that adding spices masks boar taint perception.[10] Among the different herbs and spices that have been used to mask boar taint in meat or meat products are garlic, bay leaves, clove,[11] oregano,[12] mace, marjoram, coriander, ginger and nutmeg apple.[13]

Any consideration of herbs and aromatic plants in the kitchen leads to a review of traditional practices.[14] "Mediterranean" cooking includes not only the use of different types of ingredients and spices e.g. garlic, parsley, pepper, nutmeg, olive oil, vinegar, etc., but also certain processing methods such as marinating or breading. Marinating, ("adobo" in Spain), is a traditional way of cooking that has been used since the XVIII century, the principals ingredients being vinegar, paprika and oregano. Breading is also an extensively used strategy, not only in Spain, but also in many other countries around the world. Breaded fried foods are favored by consumers over other culinary treatments because of the increased palatability provided by a soft and moist interior and a porous outer crispy crust.[15]

For generations, many explorations have been undertaken and geographical discoveries made in attempts to meet the desire for new flavors, tastes, and medicinal plants, or to find new markets for those already known.[14] Some typical spices from eastern countries, such as curry or fennel, have been incorporated into European cooking.

As regards the problem of boar taint, if castration is prohibited, the market situation will change, and it will be necessary to identify masking strategies that will still provide high quality products.[12] Works that study the use of masking methods in fresh pork from entire male pigs are scarce compared to those dedicated to processed products, meaning that market studies of tainted meat from extra and first quality cuts, such as loin, are needed. It was therefore thought interesting to investigate the effect of different masking strategies involving the addition of mixed spices or fennel, marinating, or breading

(garlic-parsley or curry) on AND odor and flavor perception to increase the quality of meat from entire male pigs.

EXPERIMENTAL

The effect of different masking strategies on loin chops with high levels of androstenone, (1.0-2.9 mg kg $^{-1}$ AND in fat) and the equivalent cuts from castrated animals (<0.4 mg kg $^{-1}$ AND), both with low levels of skatole (<0.1 mg kg $^{-1}$ SKA in fat) [1] were investigated (Borriser-Pairó *et al.*[1]).

To select the best masking strategies to reduce AND perception, a sensory evaluation of different masking strategies for the loin samples was carried out by a trained panel.

Animals and meat samples analyzed

Pigs were selected from a prior study carried out by Borrisser-Pairó *et al.*[1] The fat content of *Longissimus thoracis et lumborum* muscle was analyzed using petroleum ether (40–60 °C) with a Soxhlet unit, following AOAC guidelines.[16] Meat samples of *Longissimus thoracis et lumborum* muscle from pigs with less than 0.1 mg kg^{-1} SKA and <0.4 mg kg $^{-1}$ AND (three castrated males pigs) and animals with 2.3-2.6 mg kg^{-1} AND in fat tissue (back fat) (three entire male pigs) were used. Loins were sliced, vacuum packaged and frozen 24 h *post-mortem* and stored at -18 ºC.

To evaluate the masking capacity of the strategies, eleven groups were established, depending on whether the meat used was from entire (E) or from castrated (C) animals and with (+) or without (-) masking strategy (cooking with spices or breadcrumbs and marinating).

E-: meat from entire male pig with no masking strategy.

C+: meat from castrated pigs subjected to masking strategy in order to evaluate the similarity between the organoleptic characteristics of treated meat from entire and

castrated animals, understanding meat from castrated animals as the optimal sensory objective.

E+: meat from entire male pigs subjected to masking strategy in order to reduce AND perception.

Sample preparation

Before applying each masking strategy, samples were thawed for 12 h at 4 ºC. Loins from castrated and entire male pigs were prepared one hour before each session (except in the case of marinating), using the formulations detailed in Table 1.

As regards the mixed spice and fennel strategies, half of each mixture was sprinkled homogeneously on each surface of the chop. In the case of the garlic-parsley breadcrumbs recipe, chopped and minced garlic and parsley were mixed with the bread crumbs and salt in a blender (Classic, Moulinex ®, Barcelona, Spain) and the loin was flipped 6 times in the mixture to fix the covering. The same process was followed for curry breadcrumbs. Finally, for marinating, loins were left in the marinade for 24 hours and kept refrigerated at 4 ºC until they were cooked.

Cooking

All the prepared samples were fried since a previous study comparing cooking methods showed that frying reduced AND perception.[17] A 26 cm diameter frying pan and 40 mL of extra virgin olive oil (Koipe, Andújar, Spain) were used. The pan was preheated each time for 2 minute (oil temperature 150 ºC) on an induction cooking top (PI4750, Obergozo, Murcia, Spain). The meat was turned every minute until the internal temperature reached 83 ºC, as measured with a portable T200 thermometer (Digitron Instrumentation Ltd., Hertford, United Kingdom). Samples were served immediately after cooking and were tasted at 60 ºC. [18]

Panel and training

Eight panelists were chosen from the University of Murcia staff, all experienced in the profile assessment of different meat products (two men and six women aged 24 to 50 years) and sensitive to AND. The panel was trained according to ISO.[19] The screening process, length, training, validation and monitoring were as described by Garrido *et al*.[20] The sensory evaluation of meat was conducted in a laboratory (isolated from external influences, under white light and ambient temperature) with individual booths according to ISO[21] using a 10 cm unstructured scale. The samples were wrapped in aluminum foil and coded with a three digit number. In every session, the sample order presentation was balanced to account for carryover effects.[18] Mineral water (Montepinos, Soria, Spain) and unsalted bread (Aliada, Madrid, Spain) was given between samples for palate cleansing. Panelist waited one minute between samples. During the first two training sessions, each of the panelists evaluated separately two meat samples with known AND concentrations from each treatment. Afterwards, the group discussed their findings openly with the intervention of the panel leader as a moderator. The selected descriptors were intensity of meat odor, spice odor, AND odor, meat flavor, spice flavor, AND flavor, juiciness and hardness, all analyzed and rated from 0="not perceivable" to 10= "extremely perceivable".[20] Six replicates per assessor per type of animal (castrated vs. entire) x treatment were evaluated in eleven sessions. Each panelist tasted a total of six samples in each session. Sessions were carried out at 10:00 and 16:00 h.

Statistical analyses

The differences between meat from castrated and entire male pig with or without masking strategy were analyzed using an ANOVA (SPSS 15 software package, SPSS, Chicago, IL). No random effects were included in the model. When significant differences were found, means were compared by Tukey's test. Differences were

considered significant at the P < 0.05 level. Pearson correlation coefficients were evaluated to describe the relationship between fat and AND content.

RESULTS

Flavor and odor

There were significant differences for meat odor and flavor ($P < 0.001$), (Figure 1). As expected, samples from the E- group had higher meat odor values (typical odor of pork) than C+ and E+ samples ($P < 0.05$). The mean values obtained for meat odor for each strategy (including castrated and entire) were greater for fennel than for marinating and garlic-parsley breadcrumbs. The perceived intensity for odor was lower than for flavor. In general, all samples from C+ and E+ groups presented no significant differences for meat odor and flavor values ($P > 0.05$), except in the case of mixed spice ("mix"), where meat from castrated (C-) animals obtained higher values ($P < 0.05$) for meat flavor than the meat from entire animals (E+).

Figure 2 shows the results for spice and AND odor and flavor. There were significant differences for all these parameters ($P < 0.001$). For each masking strategy, values for spice odor and flavor were not significantly different between C+ and E+. All the treated samples (C+ and E+) had higher values for spicy odor and flavor than E- group. The AND odor was reduced with all methods ($P < 0.001$). The AND flavor was lower in E+ than in E- group, but higher than in C+ ($P < 0.05$).

Texture

There were differences (P < 0.05) in hardness and juiciness between the meat from entire (E- and E+) and castrated animals (C+). Perceived hardness was significantly higher and juiciness lower in E+ and E- than in C+, except in the case of juiciness in the garlic-parsley breadcrumb group (P > 0.05, Figure 3).

DISCUSSION

Flavor and odor

The differences in meat flavor observed between samples from castrated and entire animals in the mixed spices group were probably due to the high fat content of the meat from the castrated animals. Fat is a precursor of the volatiles responsible for meat odor and flavor during cooking, and several hundred volatile compounds derived from lipid degradation reactions have been found in cooked meat.[22, 23] On the other hand, AND odor could be related with lower meat odor values. Bañón *et al.*[8] confirmed that samples from entire animals lose the typical aroma and taste of meat due to boar taint, the loss being more marked in cooked meat than in dry-cured meat.

The AND odor was totally masked by all masking strategies since no significant differences were found between the meat samples from entire and castrated animals. However, AND flavor was detected by panelists in the E+ samples. Similar results were found by Tørngren *et al.*[24] who suggested that the sensory perception of Danish flank roll, manufactured from entire male pigs with less than 2.1 μg AND.g^{-1} of neck fat and using a spice mixture, was not significantly different from the values perceived for castrated pigs. Samples with garlic-parsley breadcrumbs had the highest values for spice odor and flavor, perhaps as a result of the odorous capacity of garlic. Lunde *et al.*[12] also used garlic in a marinade formula prepared to mask boar taint, but, by contrast, they found that the use of garlic was not sufficient to mask boar taint, although samples from that study had higher levels of SKA than the meat analyzed in this research. Garlic (*Allium sativum* L.) is one of the most widely used spices in cookery to complement and enhance the flavor of meat products, because of its high abundance of sulfur compounds.[25] However, the content of allicin and other flavor components and flavor precursors in garlic varies among different plants,[26] which could explain the differences in the results found by Lunde *et al.*[12] and the present study. Therefore, the addition of

garlic could mask the odor or flavor characteristics of boar tainted meat. Fresh parsley could also contribute to masking boar taint.

The other strategy that obtained high levels for spice odor and flavor and low levels for AND odor was the marinade which contained oregano, similarly to that described by Lunde *et al.*,[12] although they used an oregano extract. The flavor of oregano depends on several different compounds, and can be discriminated by the relative contents of p-cymene, c-terpinene, cis- and trans-sabinene hydrate, borneol, terpinen-4-ol, a-terpineol, thymol and carvacrol.[12] The same authors found that assessors were not able to clearly differentiate between high and low skatole samples when the samples were marinated in oregano. McCauley *et al.*[27] found that sweet and sour marinades did not totally mask the boar taint of pork cooked in the oven, although the intense odor and flavor of the marinades confused the assessors. In a study of rabbit meat masking, Petracci and Cavani[28] remarked that soaking in vinegar, oils, or both, in combination with spices, improved meat flavor or, at least, masked off-flavors.

The fennel strategy also obtained lower values for spice odor and flavor than garlic-parsley breadcrumbs ($P < 0.05$), but the masking effect on AND perception was similar ($P > 0.05$) to that obtained with the other strategies. No studies have been found in which fennel was used to mask androstenone, but fennel is known to add flavor and mask fatty, fishy odors, since it activates glomeruli in the surrounding clusters and was seen to suppress the alkylamine-induced and acid-aldehyde-induced responses of mitral cells, suggesting that the odor masking is mediated, in part, by lateral inhibitory connections in the odor maps of the olfactory bulb.[29] Finally, curry also reduced AND odor and flavor perception. Curry produces a pungent sensation, which can be divided into warm (disseminated only through the mouth) and sharp (which stimulates both the nasal and oral cavity mucous membranes).[30] According to Kenji and Mitsuo,[31] the

deodorizing effect of spices may be achieved via isothiocyanate compounds, which are the flavor and pungent components of mustard and wasabi, for example. These compounds stimulate mucus secretion in the nasal cavity, paralyzing sensory functions, which could explain the reduction in AND perception.

The garlic-parsley breadcrumbs was the most effective strategy (72 % reduction in AND flavor), followed by marinating (69 %), fennel (67 %), curry breadcrumbs (66 %) and mixed spice (48 %).

Texture

The loins from entire male pigs had higher values for hardness, perhaps due to the lower content of intramuscular fat compared to castrated animals.[32] In general, greater hardness is related with low juiciness perception. In addition, entire pigs could behave more aggressively during transport to the slaughterhouse than castrated animals, and this fact could increase the DFD (dark, firm and dry) meat.[33] Pauly *et al.*[34] found that the tenderness of pork from barrows was higher than for pork from entire male pigs. Font i Furnols *et al.*[35] reported that pork from entire male pigs received lower juiciness values but also lower tenderness values than pork from barrows, and intermediate values for immunocastrates. For their part, Pauly *et al.*[34] indicated that, the fat deposition differences observed in the carcasses affected the intramuscular fat percentage and so the tenderness values. Bañón *et al.*[36] found no clear effect of castration on juiciness or tenderness. The meat of castrated pigs was juicier and more tender than meat from entire pigs only when there were high amounts of intramuscular fat in the castrated pigs.

A lower value for hardness was expected for the marinating strategy, since marinades are acid liquids. Meats are marinated primarily to flavor them and to make them more moist and tender, since the acid weakens muscle tissue.[38] The garlic-parsley

breadcrumbs strategy was the only one that presented no differences between the scores for the meat from entire and castrated animals, obtaining higher juiciness values than all the other groups. In breaded fried foods, the combination of fat in the crust layers and moisture in the core affects the juiciness of the product when released inside the mouth. Juiciness in breaded fried food can be defined as the amount of juice released during consumption.[39] The layer of dry breading buffers the meat surface from direct contact with the oil. This layer quickly dries out into a pleasingly crisp surface, and forms a poorly conducting matrix of dry starch with pockets of steam or immobilized oil so that meat juices cease to flow.[38] Therefore, garlic-parsley breading could be a good option to balance the lower fat content of entire meat samples by adding juiciness.

Previous studies obtained higher values for fat in castrated animals than in entire animals (Serrano *et al.*[40]). In the present study the relation between AND concentration and the fat content obtained a Pearson correlation score of -0.747 ($P < 0.01$, n=15) (data not shown). This is because castration increases intramuscular fattening of the meat.[41] In a meta-analysis, Pauly *et al.* [34] found that carcasses of entire male pigs were leaner and the intramuscular fat content of the *Longissimus dorsi* was lower than in castrated and immunocastrated pigs. Adipocyte metabolism is influenced by sex steroids (primarily testosterone in entire males pigs, which is synthesized by the testes as AND) which affect gene transcription by binding to the nuclear Zn-finger transcription factor that recognizes steroid response elements. Castrated male rats exhibited decreased lipolysis probably as a result of defective adenylyl cyclise catalysis and the decreased number of β-adrenergic receptors, again implying desensitization to cathecolamines, and so greater fat deposition.[42]

CONCLUSION

The masking strategies used (fennel, mix, garlic-parsley breading, curry breading and marinating) can be considered as possible options to reduce the AND odor and flavor in fresh pork. The most suitable strategy would be the garlic-parsley breadcrumbs as reflected in the diminished perception of AND and also the hardness of the meat. Although the treatments helped reduce AND perception, this attribute was always significantly higher in E+ than in C+ meat. In addition, breadcrumbs seem to preserve the juiciness of the product. The results increase our knowledge of AND perception in pork meat and identify useful strategies that could be applied to the meat from entire pigs if consumers are sensitive to androstenone.

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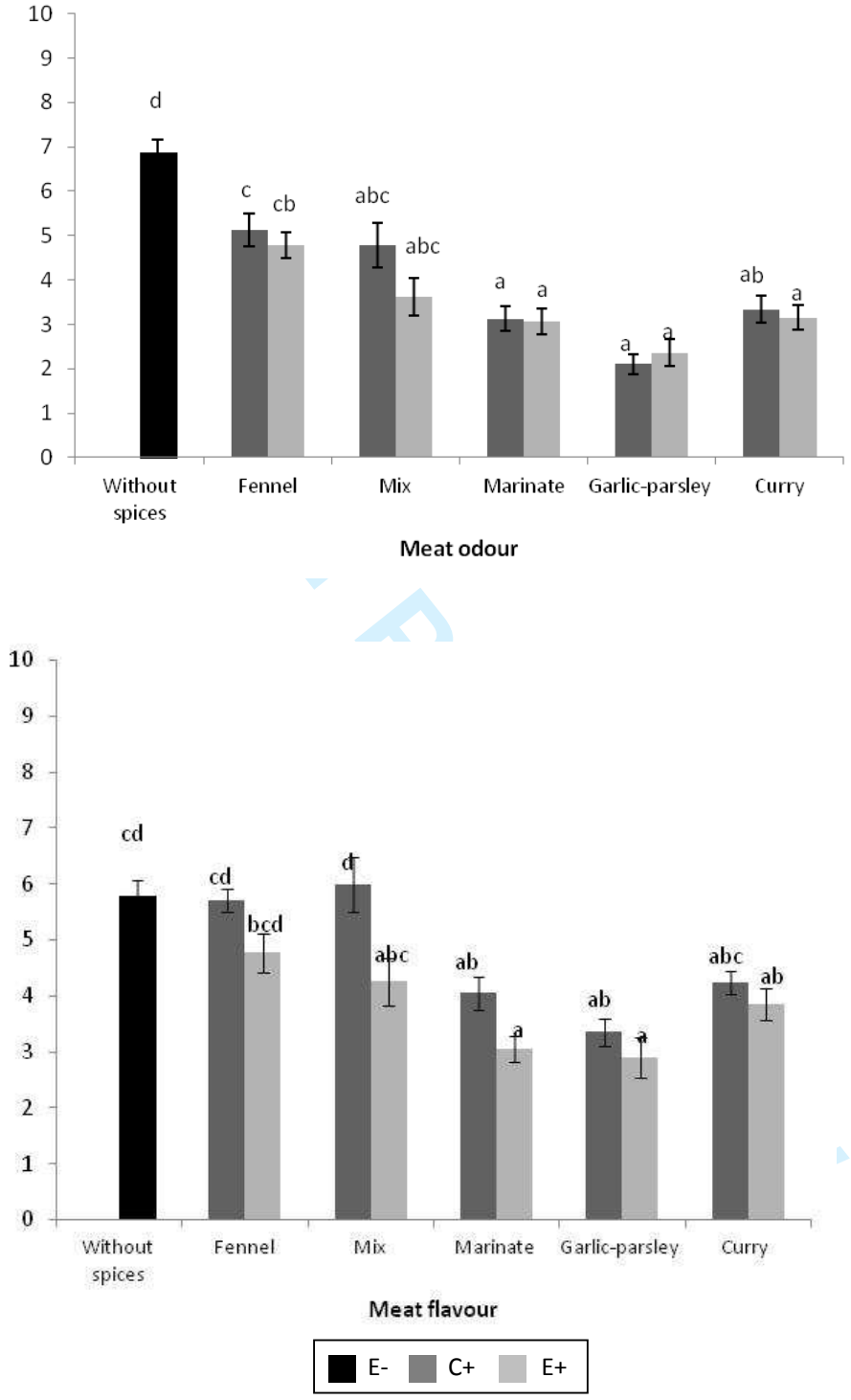

Figure 1. Mean values (10 cm scale) of meat odor and flavor analyzed from meat samples of castrated or entire animals cooked with different masking strategies (spices: fennel, mix and marinate or breadcrumb: garlic-parsley and curry). Meat from: E-: Entire male pig without processing method; C+: Castrated pigs with processing methods; E+: Entire male pig with processing methods. a, b, c, d: P<0.05 Tukey´s test.

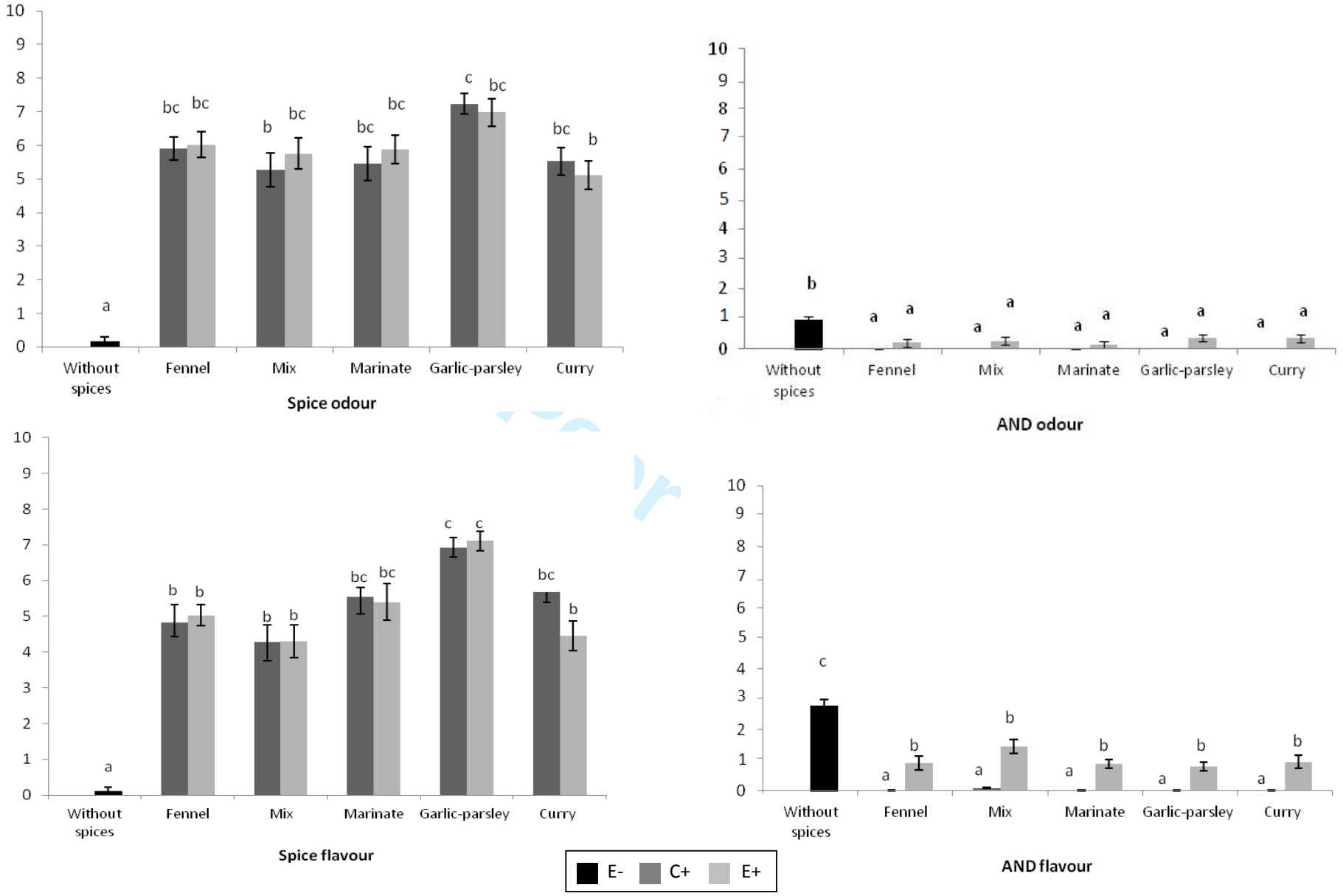

Figure 2. Mean values (10 cm scale) of spice and AND (androstenone) odor and flavor analyzed from meat samples of castrated or entire animals cooked with different masking strategies (spices: fennel, mix and marinate or breadcrumb: garlic parsley and curry). Meat from: E-: Entire male pig without processing method; C+: Castrated pigs with processing methods; E+: Entire male pig with processing methods. a, c, c, d: $P<0.05$ Tukey´s test.

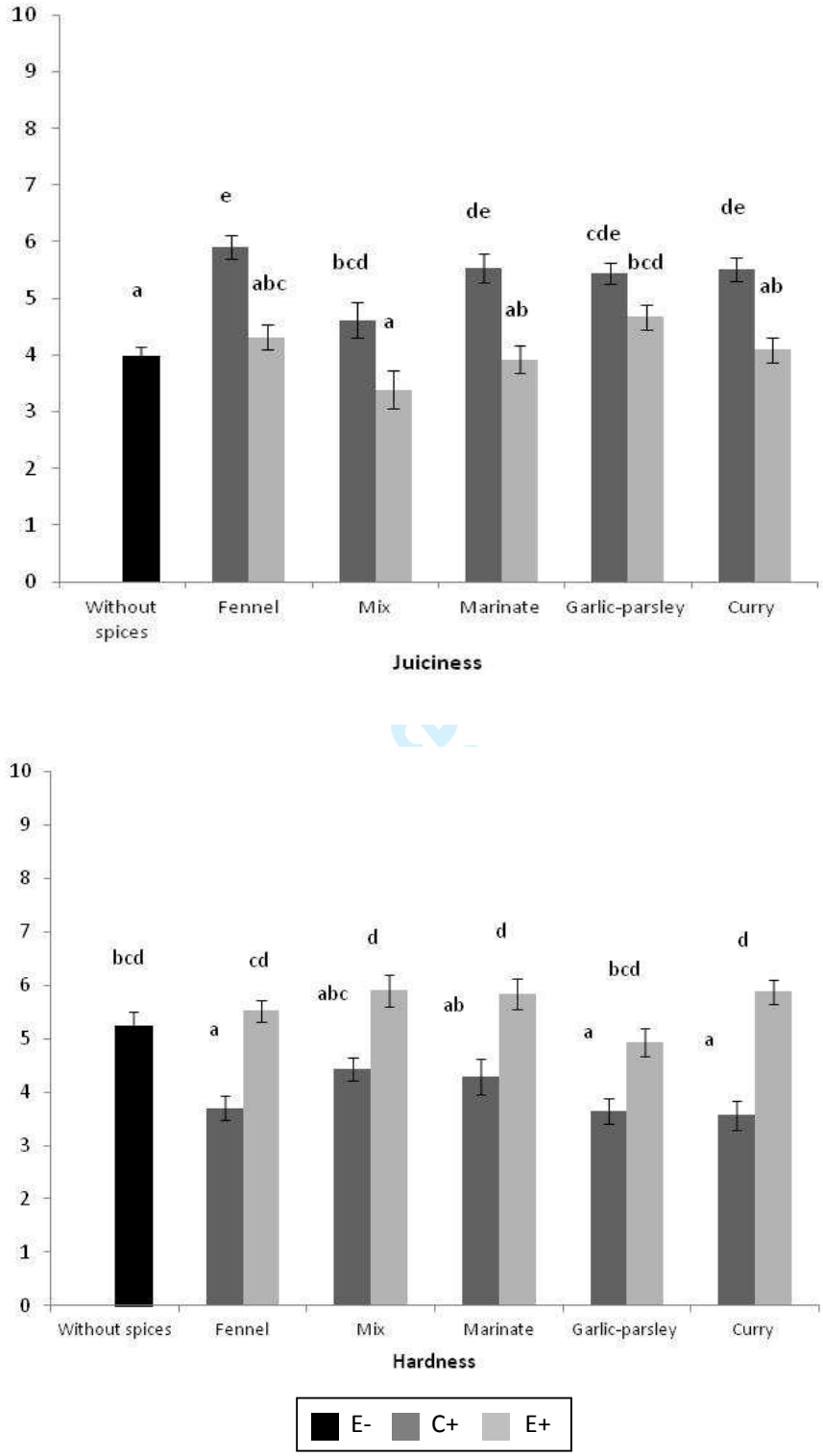

Figure 3. Mean values (10 cm scale) of texture parameters analyzed of meat samples from castrated or entire animals cooked with different masking strategies (spices: fennel, mix and marinate or breadcrumb: garlic-parsley and curry). Meat from: E-: Entire male pig without processing method; C+: Castrated pigs with processing methods; E+: Entire male pig with processing methods. a, b, c, d: $P<0.05$ Tukey´s test.